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The MNS16A polymorphism in the *TERT* gene in peri-centenarians from the Han Chinese population

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MNS16A, a variable number of tandem repeats polymorphism in the *TERT* gene, has been suggested to regulate telomerase activity. As telomerase activity has been reported to be related to life-span, we hypothesized that this polymorphism might affect human longevity by controlling the length of the telomere. To test this hypothesis, we collected 446 unrelated peri-centenarian individuals (age≥90, mean 94.45±3.45 years) and 332 normal controls (age 22–53, mean 35.0±12.0 years) from Dujiangyan, Sichuan, China. We typed the MNS16A polymorphism in both groups, and compared the allele and genotype frequencies between the peri-centenarian and control groups using the chi-squared test. There was no significant difference between the peri-centenarian and control groups. Thus, the MNS16A polymorphism in *TERT* might not influence human life-span, at least in the Han Chinese population studied here.

telomerase, longevity, MNS16A polymorphism, peri-centenarian

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Aging is a complex physical process that encompasses a variety of changes in many bodily functions, such as decreased cellular proliferative potential, a less efficient immune system, and changed endocrine system functions [1–3]. During aging, genetic and environmental factors interact with each other and both provide major contributions. Genetic factors are particularly crucial in the aging process and could greatly influence the life-span [4–6]. Mitchell et al. [7] estimated a 25% heritability for life-span in an Amish population from Lancaster County, Pennsylvania, USA.

Telomeres are structures that cap the distal ends of chromosomes, protecting them from degradation, end-to-end fusions, rearrangements, and chromosome attrition [8]. The length of the telomere, which is maintained by telomerase, determines the proliferative potential of cells [9,10]. Telomerase is a reverse transcriptase that elongates the TTAGGG repeats of telomeres and thus maintain the ends of chromosomes and the proliferative potential of cells [11,12]. Intriguingly, high telomerase activity, resulting in lengthened telomeres, was reported to be related to longer life-spans in mice [13] and in birds [14]. Furthermore, it has been suggested that high human telomerase activity is associated with better maintenance of telomere length, which may confer healthy aging and exceptional longevity in humans [15,16].

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Telomerase is a holoenzyme, composed of an RNA component, a catalytic protein subunit, and other telomerase-associated proteins [17]. Its catalytic subunit (telomerase reverse transcriptase, TERT) is the core component responsible for its enzymatic activity [18], and the expression level of TERT is closely associated with telomerase activity [19,20]. It can be speculated that functional polymorphisms in the *TERT* gene might be biological candidates related to longevity by influencing the expression of TERT.

Recently, a variable number of tandem repeats polymorphism, MNS16A, located downstream of the human *TERT* gene on chromosome 5p15.33, was reported to have an effect on *TERT* expression and telomerase activity [21]. The region containing MNS16A was found to have promoter activity on *TERT* expression, and the promoter-like activity was influenced by the length of the MNS16A tandem repeat. Thus, the MNS16A length polymorphism could influence the activity of telomerase by regulating the expression of *TERT* [21].

We hypothesized that the MNS16A polymorphism might be a genetic factor related to longevity. To test this hypothesis, we performed an association study in a Han Chinese population.

1 Materials and methods

1.1 Subjects

A total of 446 unrelated peri-centenarian individuals (age≥90, mean 94.45±3.45 years) were recruited from Dujiangyan, Sichuan, China. As reported in our previous studies [22–24], the age of the subjects was certified by the Chinese Fifth National Census, and was also supported by the number of generations of their offspring (≥3), and local village records. Only subjects whose age was supported by the government identity record and local village record, and who fulfilled the required number of offspring generations, were included. The demographic characteristics of the individuals have been described in our previous work [22–24]. At the same time, we recruited 332 healthy local younger people from the same area (age 22–53, mean 35.0±12.0 years) to be normal controls. All participants signed informed consent. This research was approved by the Ethics Committee on Human Experimentation of Kunming Institute of Zoology, Chinese Academy of Sciences. The study was performed in accordance with the Declaration of Helsinki and its subsequent amendments.

1.2 Polymorphism genotyping

Peripheral blood was collected from the participants and genomic DNA was extracted using the standard phenol/chloroform method. The MNS16A polymorphism was genotyped by PCR with primers as previously described [21]: forward primer: 5′-AGGATTCTGATCTCTGAAGGGTG-3′; reverse primer: 5′-TCTGCCTGAGGAAGGACGTATG-3′; GenBank: AF128894. 1. Briefly, PCR was performed in a total volume of 20 μL with an initial denaturing step of 5 min at 95°C, followed by 35 cycles of 30 s at 95°C, 45 s at 61°C, and 1 min at 72°C, and a final extension step of 10 min at 72°C. The PCR products were separated on an ethidium bromide-stained 3.0% agarose gel, visualized with UV light, and photographed. Ten percent of the samples were re-genotyped and subjected to direct sequencing to confirm the accuracy of the genotyping.

1.3 Statistical analysis

The genotype and allele frequencies in both groups were compared by Pearson's chi-squared test. Fisher's exact test was applied when there were low counts (<5) in the data. The *P*-value is two-tailed. When the *P*-value was <0.05, it was considered to be statistically significant and was further subjected to Bonferroni correction for multiple testing. Analyses were performed using SPSS software (version 17.0, SPSS Inc., Chicago, IL, USA). A test for Hardy-Weinberg equilibrium for the MNS16A polymorphism in the peri-centenarian and control groups was performed using GENEPOP software [25].

2 Results and discussion

We genotyped the MNS16A polymorphism in the *TERT* gene in 446 peri-centenarians and 332 matched young individuals. We observed three length variants at this locus: 243, 272, and 302 bp (Figure 1), which together formed five genotypes (Table 1). The genotype frequencies in the peri-centenarian and control populations did not deviate from Hardy-Weinberg equilibrium ($P>0.05$), in accordance with our previous work [22–24].

The proportion of homozygote and the genotype and allele frequencies of MNS16A are listed in Table 1. The most common genotype was 302/302, followed by 302/243, 302/272, 272/243, and 243/243. The 302-bp allele had the highest frequency of 94.28% in the longevity group, with a very similar frequency (93.67%) in the control group. Interestingly, we did not observe the previously reported 333-bp

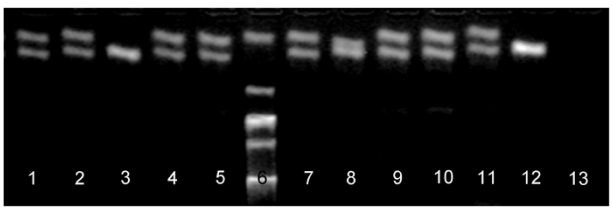

Figure 1 Genotype patterns of *TERT* MNS16A. The different MNS16A allele lengths were named according to their PCR fragment size. Lanes 1, 2, 4, 5, 7, 9–11: 302/243; lanes 3 and 12: 302/302; lane 6: DNA marker; lane 8: 302/272; lane 13: negative control.

Table 1 Genotype frequency, allele distribution, and homozygote proportion for MNS16A in the peri-centenarian and control groups

Genotype	Peri-centenarian (*N*=446)		Controls (*N*=332)		*P* value
302/302	397	89.01%	293	88.25%	0.74
302/272	18	4.04%	17	5.12%	0.47
302/243	29	6.50%	19	5.72%	0.65
272/243	1	0.22%	1	0.30%	0.83
243/243	1	0.22%	2	0.60%	0.40
Homozygotes	398	89.24%	295	88.86%	0.87
Heterozygotes	48	10.76%	37	11.14%	0.87
Alleles					
243	32	3.59%	24	3.61%	0.98
272	19	2.13%	18	2.71%	0.46
302	841	94.28%	622	93.67%	0.62

Table 2 Genotype frequency, allele distribution, and homozygote proportion for MNS16A in the peri-centenarian and control groups, stratified by gender

Genotype	Peri-centenarian female (*N*=322)		Control female (*N*=149)		*P* value	Peri-centenarian male (*N*=124)		Control male (*N*=183)		*P* value
302/302	288	89.44%	137	91.95%	0.28	109	87.90%	156	85.25%	0.51
302/272	12	3.73%	6	4.03%	0.87	6	4.84%	11	6.01%	0.66
302/243	21	6.52%	5	3.36%	0.16	8	6.45%	14	7.65%	0.69
272/243	0	0.00%	1	0.67%	0.14	1	0.81%	0	0.00%	0.22
243/243	1	0.31%	0	0.00%	0.50	0	0.00%	2	1.09%	0.24
Homozygotes	289	89.75%	137	91.95%	0.45	109	87.90%	158	86.34%	0.69
Heterozygotes	33	10.25%	12	8.05%	0.45	15	12.10%	25	13.66%	0.69
Alleles										
243	23	3.57%	6	2.01%	0.20	9	3.63%	18	4.92%	0.44
272	12	1.86%	7	2.35%	0.62	7	2.82%	11	3.01%	0.89
302	609	94.57%	285	95.64%	0.49	232	93.55%	337	92.08%	0.49

allele [21,26], possibly because it is rare in human populations [21]. In a previous study in Chinese population, the frequency of the 333-bp allele was only 0.06% in 798 cases and 0% in 1019 control individuals [27].

We found no significant differences between the longevity group and the younger control group in terms of proportion of homozygotes, genotype distribution, or allele frequency (all *P*-values>0.05; Table 1). To investigate any potential effect of gender, we divided the two groups into four: female peri-centenarian, male peri-centenarian, female control, and male control. All groups were in Hardy-Weinberg equilibrium, and there were no significant differences in genotype distribution or allele frequency when stratified by gender (all *P*-values>0.05; Table 2).

It is well established that the *TERT* gene and its MNS16A polymorphism play an important role in regulating telomerase activity [21]. In addition, different MNS16A lengths are reported to be associated with cancer risk [21,27–29]. However, aging is a very complex process, and the role of telomerase during aging might be different from that in tumorigenesis. This might explain why we did not find an association between the MNS16A polymorphism and longevity.

Even though we included 778 individuals in the present study, it is possible that this sample size is insufficient to detect a significant association in the Chinese population. If this is the case though, it indicates that variation at MNS16A has a minimal effect on the aging process in the Chinese population. Further studies on *TERT* and other genes that determine telomerase activity, such as *TERC*, are needed to understand the role of telomerase in the longevity of human populations.

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